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How does the polymorphism of the *PRL*, *PRLR*, and *RYR1* genes influence the selected reproduction traits in the Polish Large White and the Polish Landrace sows

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The objective of the research was to analyze the polymorphism of the prolactin gene, prolactin receptor, and ryanodine receptor, as well as to determine its effect on the reproduction traits of sows. The genetic examinations were done using the PCR-RFLP method. The research was conducted on 88 Polish Large White sows and 27 Polish Landrace sows, taken from broodstocks. The genetic structure was analyzed in terms of the examined restriction fragments, depending on breed, and the mean number of piglets reared alive until day 21 of their life in three subsequent farrows was determined. Two *RYR1* genotypes were observed in the examined group of sows, and three of both *PRL* and *PRLR* genotypes. Considering the number of piglets born and reared alive until the age of 21 days in three subsequent farrows, it was concluded that in the Polish Large White and Polish Landrace sows these traits were similar.

KEY WORDS: polymorphism / *PRL, PRLT, RYR1* genes / reproduction traits / sows

Improving the reproductive performance of pigs is currently one of the most essential objectives of breeding. It is directly related to the increase of economic requirements in this branch. In recent years, the research aimed at detecting genetic conditioning of reproductive traits in sows (with low heritability index) have focused on searching mutations within several dozen selected genes [2, 4]. In the improvement of the reproductive traits in sows, the most important are those genes and their metabolites which become active in various parts of the female reproductive organs. If we determine the effect of the polymorphism of the examined genes on the size of particular farrows, we may then use them as selective indices for reproductive performance in sows [6, 11].

The objective of the research was to determine the allele and genotype frequencies of prolactin, prolactin receptor, and ryanodine receptor, as well as to analyse their effect on selected reproductive traits in sows kept in four broodstocks in the Kujawsko-Pomorskie province.

Material and methods

The research was conducted on 115 animals, including 88 Polish Large White sows and 27 Polish Landrace sows from four broodstocks (in Wronie, Michałowo, Słupy Duże, and Borzymie) kept by the Kujawsko-Pomorskie division of the POLSUS Polish Pig Breeders and Producers' Association. The number of animals in each broodstock was similar, and it was equal to 30, 30, 30, and 25 respectively. The animals were kept in sow stalls with flat floor and no bedding, adopted for loose and in-pig sows.

The genomic DNA being the material for further analysis was isolated from full peripheral blood, in accordance with the protocol devised by the manufacturer, and with our own modifications added, using the Master Pure DNA Purification kits (Epicentre Technologies). We conducted both a quantitative and qualitative analysis of the isolated DNA. The amount of DNA was at the level of 100-150 ng/µl with 90% purity. The genotypes of prolactin, prolactin receptor, and ryanodine receptor were determined by means of the PCR-RFLP method, using specific oligonucleotide sequences (*PRL* according to Babicz et al. [1], *PRLR* according to Drogemüller et al. [6], *RYR1* according to Fuji et al. [7] (Sigma Aldrich) [1, 6, 7]. The reactive mixture for each of the genes contained 100 ng of the genomic DNA, 10 pmol of each of the starters, 200 µm of dNTP's, 1U Dream Taq Polymerase (MBI Fermentas). The thermal conditions of the reactions for particular genes were as follows: *PRL* – preliminary denaturation at 95°C for 3 minutes, then 35 proliferation cycles at 94°C – 45 seconds, 58°C – 30 seconds, and 72°C – 120 seconds, and final elongation at 72°C for 5 minutes; *PRLR* – preliminary denaturation at 94°C for 4 minutes, then 35 proliferation cycles at 94°C – 30 seconds, 55°C – 60 seconds, and 72°C – 30 seconds, and final elongation at 72°C for 5 minutes; *RYR1* – preliminary denaturation at 95°C for 1 minute, then 40 proliferation cycles at 95°C – 45 seconds, 58°C – 120 seconds, and 72°C – 120 seconds; and final elongation at 72°C for 5 minutes. The PCR products with the length of 298 bp (*PRL*), 163 bp (*PRLR*), and 134 bp (*RYR*1) underwent restriction enzyme digestion, respectively *Taa*I at 65°C for 2 h, *Alu*I and *Hha*I at 37°C for 4 h (MBI Fermentas), and then fragments were separated electrophoretically in 3% agarose gel containing ethidium bromide, they were visualised in UV light, and finally identified against the pUC19/*Msp*I molecular marker (MBI Fermentas) [1, 6, 7, 11, 15].

Based on the obtained results, we determined the genetic structure of the analysed sow groups with regard to the examined restriction sites in accordance with the Hardy-Weinberg principle, taking into consideration which breeds the sows came from [5]. We then used the chi-square test to verify the distribution of the genotype frequencies established by means of the Hardy-Weinberg principle [5, 18]. Taking into account the genotypes and breeds of the sows, we calculated the mean number of piglets born and reared alive until the age of 21 days in three subsequent farrows.

In order to determine the effect of the genotype on the examined reproductive traits in sows, we applied the multivariate analysis of variance and the Duncan test. We used the Statistica 8.0 ANOVA software to analyse the results statistically.

Results and discussion

The information on genetic conditioning of reproductive performance in sows can be used if we analyse the polymorphisms of hormone genes and their receptors. This makes it possible to estimate the breeding value in more detail. Therefore, it is essential to determine the genotypes of the sows related to genes with a significant effect, which include *PRL*, *PRLR* and *RYR1*, as well as their reproductive performance. The prolactin gene and prolactin receptor are both genes of hormones which stimulate the lactogenesis and lactopoesis processes, the level of which rises during birth and maturation. Prolactin induces the synthesis of casein mRNA and alpha-lactalbumin, and accelerates the translation process. It also influences the rise in the lipoprotein lipase activity, stimulating the fatty acids synthesis. Whereas the prolactin receptors located in the mammary gland and the cytoplasmic membranes of the milk fat drops, at the time of being joined with the prolactin, generate a signal to start milk protein genes' activity [3, 13, 16]. Mutation in the ryanodine receptor gene C→T is related to the pigs being prone to stress, which may cause lower prolificacy, decreased libido, and in extreme cases even the death of an animal [14].

Table 1 shows the characteristics of the examined group of the Polish Large White and Polish Landrace sows, taking into consideration the frequency of alleles and the *PRL*, *PRLR*, and *RYR1* gene genotypes. In the Polish Large White sows, the allele frequencies for the *Del* (0.421) and *Ins* (0.580) prolactin genes, as well as the *A* (0.483) and *B* (0.517) alleles of its receptor, were similar, having roughly the same level, whereas the frequency of the *C* allele of the ryanodine receptor gene (0.989) was nearly one hundred percent (Table 1).

In the examined Polish Landrace sows, we observed varying allele frequencies both in the *PRL* as well as *PRLR* locus (Table 1). The distribution of allele frequencies in the *PRL* locus for the Polish Landrace sows was as follows: *Ins* = 0.370, and *Del* = 0.630. Similar results as regards the gene frequencies, including locus, were reported by Babicz et al. [1] (*Ins* = 0.34, *Del* = 0.66). Whereas in the *PRLR* locus we established the allele frequency of *A* = 0.426 and *B* = 0.574 (Table 1). Kmieć and Terman [9], having examined groups of the Polish Large White and Polish Landrace sows, found a similar frequency of the *B* allele (0.59). In the Polish Large White sows, considered to have a fairly low load related to the gene of sensitivity to the $RYR1^T$ stress, mutations of this gene occurred less frequently (0.011) as compared to the Polish Landrace sows (0.222) (Table 1). The research concerning the allele frequency of the *RYR1* gene, conducted by Janik et al. [8], showed that $RYR1^T$ frequency was between 0.11 and 0.25.

In the examined group of sows, three genotypes were noted both in the *PRL* as well as *PRLR* locus, and two *RYR1* genotypes (Table 1). In the *PRL* locus, the sows with the *Ins/Del* genotype were more common both among the Polish Large White sows (frequency of 0.432) and the Polish Landrace sows (frequency of 0.519) (Table 1). Babicz et al. [1], however, observed something else. They reported higher percentage of the *Del/Del* homozygotes, which was at the level of 0.54.

In the examined group of the Polish Large White, there was a higher number of animals having the $PRLR^{BB}$ genotype (frequency of 0.386), and in the Polish Landrace the $PRLR^{AB}$ genotype was more common (frequency of 0.556) (Table 1). Kmieć et al. [10], who exa-

Table 1 – Tabela 1

The genetic structure of the examined groups of the Polish Large White (PLW) and Polish Landrace (PL) sows

Struktura genetyczna badanej grupy loch ras wielkiej białej polskiej (wbp) i polskiej białej zwisłouchej (pbz)

Gene Gen	Genotype Genotyp		Breed Rasa							
			PLW – wbp (n=88)				PL – pbz (n=27)			
			n	Frequency Frekwencja		Chi^2	n	Frequency Frekwencja		Chi^2
				observed obserwowana	expected oczekiwana			observed obserwowana	expected oczekiwana	
PRLR/ Alu I	Genotype Genotyp	AA	31	0.352	0.233		4	0.148	0.181	
		AB	23	0.261	0.499	22.72	15	0.556	0.489	1.86
		BB	34	0.386	0.267		8	0.296	0.330	
	Allele Allel	A		0.483				0.426		
		B		0.517				0.574		
PRL/ TaaI	Genotype Genotyp	Del/Del	18	0.205	0.177		10	0.370	0.397	
		Ins/Del	38	0.432	0.487	1.19	14	0.519	0.467	1.25
		Ins/Ins	32	0.364	0.336		3	0.111	0.137	
	Allele Allel	Del		0.421				0.630		
		Ins		0.580				0.370		
RYR1/ HhaI	Genotype Genotyp	CC	86	0.977	0.977		15	0.556	0.605	
		CT	2	0.023	0.023	0.01	12	0.444	0.272	
		TT	-	-	0.0001		-	-	0.049	16.33
	Allele Allel	C		0.989				0.779		
		T		0.011				0.222		

Ch^2 tab. $p \leq 0.05 – 5.99$; $p \leq 0.01 – 9.21$

mined Polish Large White sows, found the $PRLR^{AB}$ heterozygotes to be the most frequent (0.4817), and the $PRLR^{AA}$ homozygotes (0.1030) were the least frequent.

The $RYR1^{CC}$ frequency was higher than that of the $RYR1^{CT}$ genotype, and for the Polish Large White sows equalled 0.977, and for the Polish Landrace 0.556 (Table 1). Similar results were obtained by Janik et al. [8], who examined pure-bred Polish Large White and Polish Landrace sows. They observed the $RYR1^{CC}$ frequency of 0.6850 and the $RYR1^{CT}$ frequency of 0.2760 in the Polish Large White breed, and the $RYR1^{CC}$ frequency of 0.4690 and the $RYR1^{CT}$ frequency of 0.4680 in the Polish Landrace. The genotype distribution for the Polish Large White sows conformed to the genetic equilibrium principle in the *PRL* and *RYR*1 locus, whereas for the Polish Large White, in the *PRLR* and *PRL* locus (Table 1).

Analysing the number of piglets born and reared alive until the age of 21 days in three subsequent farrows, we determined that these traits were similar in the Polish Large White and the Polish Landrace sows (Table 2). The number of piglets born and reared alive until day 21 was higher in the Polish Large White sows with the $PRLR^{BB}$ genotype as compared to the sows with the other two genotypes. Such clear tendency was not observed in the examined group of the Polish Landrace sows. Results different to ours were obtained by Kmieć et al. [11]. They indicated a positive effect of the *A* allele. Having analysed the Polish Landrace pigs population, they established a statistically significant difference, at $p = 0.1$, of 0.35 piglets in a farrow between animals with the $PRLR^{AA}$ and $PRLR^{BB}$ genotypes. The first farrow of the sows with the $PRLR^{AA}$ genotype had the level of 10.51, whereas the $PRLR^{AB}$ heterozygotes gave birth to 10.44 piglets in the first farrow, and the $PRLR^{BB}$ homozygotes to 10.16 piglets. Rothschild et al. [17], having examined the effect of particular polymorphic variations of the prolactin gene receptor on the increase of the number of piglets born alive in three subsequent farrows, failed to determine any statistically significant differences.

In the group of the Polish Large White sows, the three smallest subsequent farrows in terms of the number of piglets born and reared alive came from sows with the PRL^{InsDel} genotype, whereas in the group of the Polish Landrace sows with the PRL^{DelDel} genotype (Table 2).

The Polish Landrace sows with the $RYR1^{CC}$ genotype gave birth to and reared slightly more animals per a farrow than the sows with the $RYR1^{CT}$ genotype, and the differences in the number of piglets born alive were at the level of 0.22 piglets in the first farrow, up to 0.85 piglets in the third farrow (Table 2). Kmieć et al. [12], when examining the dependencies between the polymorphism in the ryanodine receptor gene and the selected reproductive performance traits in the Polish Landrace sows, found statistically insignificant differences between the $RYR1^{CC}$ homozygotes and $RYR1^{CT}$ heterozygotes. Research conducted with respect to the effect of the $RYR1^{T}$ gene on the reproductive performance, however, suggests a negative influence of this type of gene on the reproductive traits value both in the pure-bred and cross-bred sows; similarly to our own research. Farrows of sows sensitive to stress were smaller as compared to those who were immune, both at birth and on day 21 of their lives.

Our results combined with the information available in the specialist literature on the analysed polymorphic sites of the *PRL*, *PRLR*, and *RYR*1 genes suggest that these genes are related to the traits influencing the size of the subsequent farrow and piglet survival

Table 2 – Tabela 2

The effect of the polymorphism the *PRL, PRLR* and *RYR1* genotypes on the average value of the analysed traits in the Polish Large White and the Polish Landrace breeds
Wyniki użytkowości rozpłodowej loch ras wielkiej białej polskiej (wbp) i polskiej białej zwisłouchej (pbz) z uwzględnieniem polimorfizmu *PRL, PRLR* i *RYR1*

Trait Cecha		Breed Rasa		Genotype *PRLR/Alu*I Genotypy *PRLR/Alu*I						Genotype *PRL/Taa*I Genotypy *PRL/Taa*I						Genotype *RYR1/Hha*I Genotypy *RYR1/Hha*I			
				PLW wbp (n=88)			PL pbz (n=27)			PLW wbp (n=88)			PL pbz (n=27)			PLW wbp (n=88)		PL pbz (n=27)	
		PLW wbp (n=88)	PL pbz (n=27)	*AA* (n=31)	*AB* (n=23)	*BB* (n=34)	*AA* (n=4)	*AB* (n=15)	*BB* (n=8)	*Del/Del* (n=18)	*Ins/Del* (n=38)	*Ins/Ins* (n=32)	*Del/Del* (n=10)	*Ins/Del* (n=14)	*Ins/Ins* (n=3)	*CC* (n=86)	*CT* (n=2)	*CC* (n=15)	*CT* (n=12)
1st farrow – I miot																			
Number of piglets born alive	x	10.89	10.70	10.87	10.57	11.12	10.75	10.67	10.75	10.83	10.82	11.00	10.90	10.43	11.33	10.90	10.50	10.80	10.58
Liczba prosiąt żywo urodzonych	S	1.36	0.99	1.75	1.24	0.98	0.96	1.11	0.89	1.29	1.43	1.34	0.99	0.94	1.15	1.37	0.71	1.01	1.00
Number of piglets on day 21 of life	x	10.57	10.63	10.58	10.26	10.76	10.75	10.60	10.63	10.83	10.47	10.53	10.90	10.36	11.00	10.57	10.50	10.67	10.58
Liczba prosiąt w 21. dniu życia	S	1.35	0.93	1.59	1.36	1.07	0.96	1.06	0.74	1.29	1.33	1.41	0.99	0.84	1.00	1.36	0.71	0.90	1.00
2nd farrow – II miot																			
Number of piglets born alive	x	11.29	12.14	11.22	11.26	11.35	11.67	12.27	12.14	12.06	11.13	11.00	11.88	12.30	12.33	11.24	13.00	12.27	11.83
Liczba prosiąt żywo urodzonych	S	1.32	1.06	1.73	0.93	1.20	1.15	1.01	1.21	1.48	0.91	1.50	0.99	1.06	1.53	1.29	1.41	1.16	0.75
Number of piglets on day 21 of life	x	10.97	11.90	10.91	11.00	11.00	11.67	12.09	11.71	11.44	10.88	10.80	11.88	11.90	12.00	10.97	11.00	12.07	11.50
Liczba prosiąt w 21. dniu życia	S	1.12	1.22	1.35	1.00	1.03	1.15	1.14	1.50	1.03	0.87	1.38	0.99	1.37	1.73	1.13	0.00	1.28	11.50
3rd farrow – III miot																			
Number of piglets born alive	x	11.75	11.73	11.71	11.33	12.00	–	11.56	12.00	11.07	11.58	12.27	11.67	12.00	11.33	11.70	13.00	11.85	11.00
Liczba prosiąt żywo urodzonych	S	1.69	1.16	2.20	1.72	1.21	–	1.01	1.41	1.54	1.28	1.49	1.21	1.41	0.58	1.66	2.83	1.14	1.41
Number of piglets on day 21 of life	x	11.08	11.67	10.71	10.92	11.43	–	11.44	12.00	11.07	10.78	11.53	11.67	12.00	11.00	11.02	12.50	11.77	11.00
Liczba prosiąt w 21. dniu życia	S	1.34	1.23	1.53	1.56	0.99	–	1.13	1.41	1.54	1.28	1.19	1.21	1.41	1.00	1.30	2.12	1.24	1.41

until day 21 of their lives. However, no significant effect of the polymorphism of selected genes was found on the reproductive traits of the Polish Large White and the Polish Landrace sows. This could be due to small size of our material as well as no possibility to eliminate influences other than genetic ones. The results of such analyses accompanied by the use of traditional methods may constitute a useful tool used to genetically improve the number of farrows and reared piglets in different breeds. Identifying genes which influence reproductive traits in sows will also allow selecting the animals with the genotypes which may be expected to raise the number of piglets born alive and reared.

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Wpływ polimorfizmu genów *PRL, PRLR i RYR1* na wybrane cechy reprodukcyjne loch ras wbp i pbz

Streszczenie

Celem przeprowadzonych badań była analiza polimorfizmu genu prolaktyny, receptora prolaktyny oraz receptora rianodyny, a także określenie jego wpływu na cechy reprodukcyjne loch. Badania genetyczne przeprowadzono za pomocą metody PCR-RFLP. Materiał do badań stanowiło 88 loch rasy wbp i 27 loch rasy pbz, pochodzących ze stad zarodowych. Określono strukturę genetyczną grup rasowych loch dla badanych miejsc restrykcyjnych, określono średnią liczbę prosiąt żywo urodzonych i odchowanych do 21. dnia życia w trzech kolejnych miotach. W badanej grupie loch zaobserwowano dwa genotypy *RYR1* oraz po trzy genotypy *PRL* i *PRLR*. Analizując liczbę żywo urodzonych i odchowanych do 21. dnia życia prosiąt w trzech kolejnych miotach stwierdzono, że lochy ras wbp i pbz wykazywały zbliżone wartości tych cech.

SŁOWA KLUCZOWE: polimorfizm / gen *PRL, PRLR, RYR1* / cechy reprodukcyjne / lochy